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REVIEW ARTICLE

Biomarkers: A Review

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ABSTRACT

Biomarkers are biological measures of a biological state and play an important role in understanding the relationships between exposure to environment, the development of chronic diseases and identification of subgroups that are at increased risk for disease. The importance of biomarkers has increased tremendously because of its ability to understand the whole spectrum of disease process. Many biomarkers with potential clinical value are been developed and identified by advances in high-throughput technology. Tracking the complex pathophysiology involved in disease is possible with extensive studies of genomics, proteomics, metabolomics and transcriptomics. Along with molecular biomarkers now imaging biomarkers are gaining importance, offering earlier detection of some diseases than molecular markers and enabling practitioners to see into the body without the need for invasive procedures. In this review we summarize a comprehensive literature on biomarkers with emphasis on the development and applications of biomarkers.

KEYWORDS

Biomarkers, High-Throughput Technology, Proteomics, Genomics, Transcriptomics, Metabolomics, Imaging Biomarkers

INTRODUCTION

"A Biomarker is a characteristic that can be objectively measured and evaluated as an indicator of a physiological as well as pathological process or pharmacological response to a therapeutic intervention". Biomarker can be used as an indicator of a particular disease state or some biological state of an organism. They can be specific cells, molecules, enzymes, or hormones, genes, or gene products, complex organ functions or general characteristic changes in biological structures. Biomarkers of all types have been used by generations of physicians, epidemiologists, and scientists to study human disease and also in preclinical research and clinical diagnosis. For example, elevated body temperature is a well-known biomarker for fever. C-reactive protein (CRP) for inflammation, determination of blood pressure for stroke, cholesterol values for coronary and vascular disease, all act as biomarker and risk indicator. Various tools and technologies included in biomarkers aid in understanding the cause, diagnosis, prediction, progression, regression, or outcome of treatment of disease. Any specific molecular alteration of a cell on DNA, RNA, metabolite or protein level can be referred to as a Molecular biomarker[1,2]. Biomarkers have gained immense scientific and clinical value and interest in the field of pharmacy because it provides a dynamic and powerful approach in understanding the entire spectrum of disease from earliest manifestations

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to the terminal stages. Staging, grading, and selection of initial therapy can be determined by biomarkers during diagnosis. They can be used to monitor therapy, monitor recurrent diseases or select additional therapy. Thus, identifying biomarkers include all diagnostic tests, imaging technologies, and any other time factor and cost for phase I and II of clinical trials by replacing clinical endpoints. Advances in genomics, proteomics and molecular pathology have generated many candidate biomarkers with potential clinical value. Integration of biomarkers, identified using high-throughput technologies is used into medical practice to achieve 'personalization' of treatment and disease prevention[3,4,5].

Historical Background

Since, the very beginning of medical treatment biomarker is used to detect disease and improve treatment. Sumerian and Babylonian physicians documented urine assessments far back in 4000 BC. In those days, whenever a patient was diagnosed with severe disease, they would ask them to breathe into sheep's nose. The animal would then be slaughtered and liver was inspected for evidence of disease. The Babylonians based on this test stated that liver was the centre of the human body's organs and that the entire human physiology occurred there. The Egyptians pharaohs in the time of Cleopatra and Ikhnaton tested for pregnancy by adding a woman's urine to a bag containing wheat and barley seeds. If the seeds germinated the woman was pregnant. If the barely seeds germinated first, it was an indication that the woman was carrying a male foetus, but if wheat seeds germinated first then it indicated that the woman is carrying is female foetus. Another ancient diagnostic test was documented by utilizing the sweetness of urine and its ability to attract black ants to diagnose diabetes mellitus[6]. In the late 1980's, scientist discovered that HIV viral load could be used as a biomarker of disease advancement, and subsequently, as a part of antiretroviral treatment efficacy. The viral load biomarker was used in the development and assessment of Highly Active Antiretroviral Therapy (HAART) treatment regiments involving combination of several drugs used by many people living with HIV today[2]. The first laboratory test for cancer biomarker proteins has begun in 1847 with the discovery of Bence-Jones Protein by Henry Bence Jones in the urine of multiple myeloma patient. After one hundred and forty years it was demonstrated that the protein was also present in the serum. In the year 1998 this immunodiagnostic test was approved by FDA[7].

Table 1: Historical Landmarks in Discovery and Development of Biomarkers[1]

Year	Landmark
1847	The first laboratory test for a protein cancer biomarker, Bence-Jones Protein.
1957	The test for the measurement of transaminases in myocardial infarction.
1960s	The term 'Biomarker' started to appear in the literature in connection with meatbolics and biochemical abnormalities associated with several diseases.
1967	An improved test for myocardial infarction based on a biomarker – serum creatine phosphokinase.
1971	Report for carcinoembryonic antigen (CEA) as biomarker for cancer.
1987	Troponin I as a biomarker for myocardial infracture.
1990	Accelerator mass spectrometry used for analysis of biological samples for biomarkers.
1995	Applications of proteonomics for discovery of biomarkers and use in molecular diagnosis.
1999	Emergence of metabolomics for study of biomarkers.

2000	Sequencing of the human genome completed opening the way for discovery of gene biomarkers.
2005	Discovery and applications of biomarkers becomes a major activity in biotechnology and biopharmaceutical industry.

Classification of Biomarkers

1. Based on Characteristics

- Imaging Biomarker: An imaging biomarker is a biological aspect distinguishable in an image. For example, a simple lesion in lung detected by X-Ray, MRI, or CT can lead to the distrust of a neoplasm. The lesion itself plays as a biomarker.
- Non-imaging Biomarker: Non-imaging biomarkers are basically molecular biomarkers that have bifocal properties, which allow their measurements in biological samples. For example, cerebrospinal fluid, plasma, serum, etc.[2]

2. Based on Decision Making in Early Drug Development

- Pharmacodynamic Biomarker: Each person has different hereditary makeup; some people metabolize or change the chemical structure of drugs. Decreased metabolism of certain drugs can create dangerous conditions in which high levels of the drug assemble in the body. These biomarkers are used in selecting doses for chemotherapeutic agents in a given set of tumour patient's conditions. These markers help in enhancing cancer drug doses below their cytotoxicity level and phasing the clinical trials to next level.
- Predictive Biomarker: Predictive biomarker is defined as a marker which can be used to identify subpopulations of patients who are most likely to respond to a given therapy. It is possible to select the therapy with the highest likelihood of efficacy to the individual patient with predictive biomarkers. Thus, predictive biomarkers are the basis for individualized treatment. For example, estrogen and progesterone receptors to predict sensitivity to endocrine therapy in breast cancer[1,7].

3. Based on Drug Development

- Diagnostic Biomarker: Screening and diagnosis to detect disease at early stages can be done with these biomarkers. It also provides the means to define a population with a specific disease i.e. cardiac Troponin for the diagnosis of myocardial infarction.
- Prognostic Biomarker: They provide information on the likely course of the cancer disease in an untreated individual. It correlates with the results. For example, over expression of neu/Her-2 in breast cancer or EGFR expression in colorectal cancer indicates poor prognoses, elevated levels of metallopeptidase inhibitor 1 (TIMP 1), a marker linked with more aggressive forms of multiple myeloma[2,7,8].

4. Based on Genetic and Molecular Biology Methods

- Type 0-Natural history markers: A marker of natural history of a disease and correlates longitudinally with known clinical indices.
- Type 1-Drug activity markers: A marker that captures the effect of a therapeutic intervention in accordance with its mechanism of action.
- Type 2-Surrogate markers: Surrogate endpoints are markers of biological mechanism (i.e., biomarkers) that predict a clinical benefit, alternate for clinical endpoint, and provide a systematic view on disease (i.e., direct measurement of how a patient feel, functions, or recovers.)[7,2].

Types of Biomarkers

1. Gene Biomarker

Genes are described as blueprints of life and transmit inherited traits from one gene to another. "Gene expression" means that its DNA is used as a blueprint to produce a specific protein. Biological role of genes can be provided by the arrangements in which they are expressed.

Malfunctioning of genes is involved in most diseases. Mutations in oncogenes, tumour suppressor genes, and mismatch-repair genes can serve as biomarker. For instance, mutations in the oncogene KRAS (Kirsten rat sarcoma viral oncogene homolog) predict metastatic spread in various tumour types, and there are mutations in the gene that encode the tumour suppressor p53 in more than half of sporadic cancers[1,3].

2. DNA Biomarker

Cells contain genetic information in the form of DNA. Various types of cancer and other diseases such as sepsis and autoimmune disease are associated with DNA concentrations. Strong variation can be observed in the rate of cytosine DNA methylation between species. Several diseases, particularly cancer show DNA methylation changes where genome-wide hypomethylation coincides with gene-specific hypermethylation. Detection of cancer, classification of tumours, as well as prediction and monitoring of cancer can be done by DNA methylation patterns. As a stable nucleic acid-based modification with limited dynamic range that is technically easy to handle, DNA methylation is assuring biomarker for many applications[1,9].

3. RNA Biomarker

RNA is the earliest biological measure of a disease which provides a relevant signal of disease onset or progression before observable clinical management. Detection of oral squamous cell carcinoma can be done by profiling of human mRNA. Human mRNA is present in saliva and is used as biomarkers for oral cancer. The transcript levels of enzymes important for drug metabolism have been used preclinically to predict the response to chemotherapy in lung and colon cancers[3,7].

4. Stem Cell Biomarker

The lineages assumed by stem cells during haematopoiesis can be identified by the pattern of protein markers present on the surface of cells at different stages of differentiation. The isolation of hematopoietic stem cells has been aided by directing the specific antibodies at these markers by flow cytometry. Identification of cancer stem cells in melanoma and possibly other aggressive tumours is possible by expression of Cripto-1 which may represent as a useful marker. Endoglin, an auxiliary transforming growth factor receptor, plays an important role in angiogenesis and haematopoiesis and is functional biomarker of HSCs and neural crest stem cells (NCSCs).

Only NCSCs expressing high levels of endoglin have myogenic differentiation potential. There is reduction in expression of endoglin in NSCSs with age, with a corresponding decrease in both smooth muscle differentiation potential and TGF-β 1 responsiveness. These conclusions indicate a cell autonomous role for endoglin in smooth muscle cell specification contributing to vascular integrity[1].

5. Protein Biomarker

Most of the biomarkers in clinical use are single proteins. Protein-based 'fingerprints' may outperform individual protein markers. Protein quantity by itself might not be the salient marker parameter. Instead protein function is usually dependent on location in the cell and/or in the tissue, glycosylation, other post-translational modifications and phosphorylation. Most of the protein biomarker has been approved by USFDA.

For example, Bence-Jones protein is particularly diagnostics of multiple myelomas, carcinoembryonic antigen, CA 15-3 also known as mucin, α fetoprotein, etc.[3,7].

Characteristic of Ideal Biomarker

- An ideal biomarker should be safe and easy to measure.
- The follow-up test cost should be relatively low.
- It should be consistent across genders and ethnic groups.
- It should be sensitive and specific and have a high predictive value.
- Readily quantifiable in accessible biological fluid or clinical samples[2,3].

Development of Biomarker

Biomarker development (BMD) scientists work in partnership with transitional discovery and clinical researchers as well as external experts to provide cutting edge biomarker solutions that get the best treatment to patients quickly and with maximum benefit. They deliver integrated biomarker plans using diverse expertise and knowledge in genetics, genomics, imaging, proteomics and cellular biology, vendor scientific monitoring, statistics human tissue research and bioinformatics.

Goals of Biomarker Development

- Discover novel biomarkers.
- Evaluate the efficacy and suitability of existing biomarkers.
- Bioinformatics directed biomarkers discovery.
- Developing groups of biomarkers.

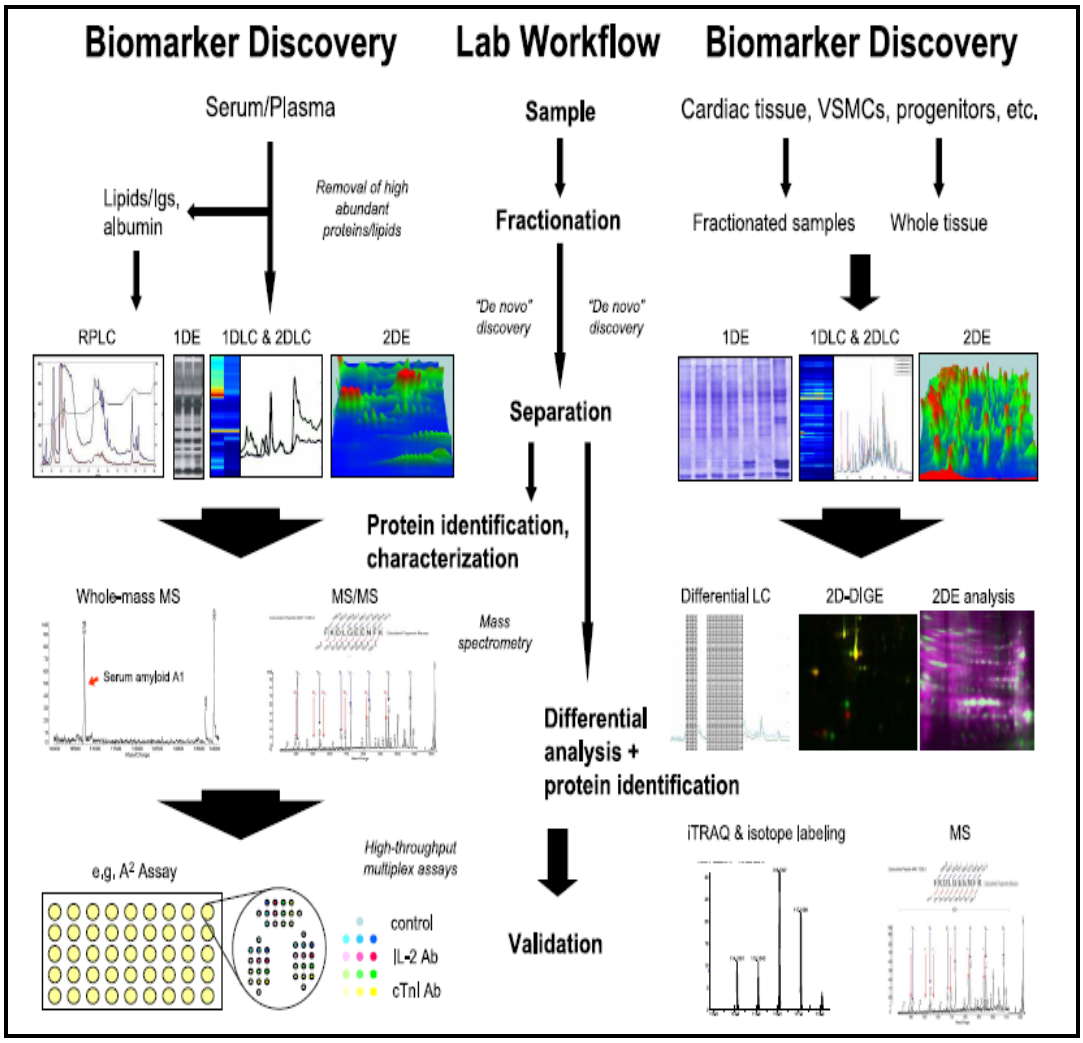

Figure 1: The Platform For Biomarker Discovery In Serum And Tissue Samples At Our Institution Combines Multiple Synergistic Protein Fractionation And Separation Methods Including One- And Two-Dimensional Gel Electrophoresis (1-DE, 2-DE), Differential In-Gel Electrophoresis (2D-DIGE), And One- And Two-Dimensional Liquid Chromatography (1DLC, 2DLC), Coupled With Mass Spectrometry (MS) Identification And Validation Methods, Using Multiplex Arrays. RPLC-Reversed Phase Liquid Chromatography, VSMC-Vascular Smooth Muscle Cell, cTnI-Cardiac Troponin I, Igs-Immunoglobulins[10]

Biomarker Discovery Using High-Throughput Technology Platforms

High-throughput screening (HTS) is a method for scientific experimentation especially used in drug discovery and relevant to the fields of biology and chemistry. Currently these technologies are used for the identification of biomarkers, as well as current approaches to increase the percentage of biomarkers that pass the barriers for clinical application.

High-throughput technologies are useful to assess:

- Genomic data defines the messages and the resulting protein sequences.
- Transcriptomic data reveals the levels of messages present.
- Proteomic data gives the levels of each protein present.
- Metabolomics data gives information related to metabolites.

1. *Genomics*

Genomics is a method in genetics that applies DNA sequencing methods, recombinant DNA and bioinformatics to arrange, assemble, and study the function and structure of genomes. The genetic messages and the resulting protein sequences are explained by genomics. Modern sequencers such as the ABI 3700 automate and multiplex the Sanger method so that it can be utilized to sequence whole genomes. Once the genome is sequenced, the next important task is the study of genetic or genome variation between individuals. Commonly considered types of variations include single nucleotide polymorphisms (SNPs) and different types of repeats. Difference between individuals especially when it comes to susceptibility to various diseases and responses to drug treatments is determined by genomic variations[3].

2. Transcriptomics

Transcriptomics is the study of the expressed mRNA transcript complement of a cell under various conditions and is possibly the best developed of the different high-throughput technologies. The major attraction in transcriptomics is that the ability to measure mRNA concentrations of all genes under any condition allows studying regulation of gene expression at a genome-wide scale as nucleic acids (such as mRNA) are much easier to separate, purify, detect and quantify than proteins. All transcription profiling methods are based on the process of hybridization, where a cDNA target from the sample to be analysed is hybridized to its complementary single stranded DNA probe on an array. The two standard technologies for transcription profiling are cDNA microarrays (where the DNA probe on the array is a long cDNA), and Affymetrix Gene Chips (where the probe on the array is a short oligonucleotide)[3].

3. Proteomics

Proteomics could be described as a large-scale study of protein structure, expression, and function including modifications and interactions. In general, two different strategies are being used to discover biomarkers using proteomic technologies. The first approach is a "targeted" path based on the traditional assumption based interpretation of specific biomarker candidates, selected from analysis of candidates obtained from other sources or on the basis of biological rationale. The second approach is a "de novo" discovery that uses distinct proteomic techniques and validates potential biomarker candidates. Both approaches have advantages and disadvantages, are complementary and may be performed in parallel. Proteomics methodologies include:

- Western blotting and ELISA and other antibody-based methods are used for the assessment of protein expression.
- The isolation, recognition, and evaluation of proteins in biosamples with high-resolution 2-D gel electrophoresis, surface chromatography by adsorption of proteins to activated surfaces (matrix-assisted laser desorption–ionization technology), high-performance liquid chromatography or via peptide ionization procedures and mass spectroscopy. A comprehensive profile of

peptides and proteins in biosamples can be achieved by Mass Spectroscopy without the need for initial protein separations, thereby speeding up biomarker identification with reduced sample requirements and a high throughput[4,11].

4. Metabolomics

It is a whole-cell measurement of all the metabolites and it is considered to be equivalent to transcriptomics in mRNA expression analysis which is referred to as 'metabolic fingerprinting'. The metabolome exhibits the collection of all metabolites in an organ, biological cell, organism, or tissue which are the end products of cellular processes. Metabolic profiling can give an instantaneous snapshot of the physiology of that cell[4].

Phases of Discovery and Evaluation of Biomarkers

In 2002, the National Cancer Institute's 'Early Detection Research Network' developed a five phase approach to systematic discovery and evaluation of biomarkers. In general, biomarker development should follow an orderly process wherein one proceeds to the next phase only after meeting pre-specified criteria for the current phase[3].

Table 2: Phases of Discovery and Evaluation of Biomarkers[3]

Phase 1	These are preclinical exploratory studies. Selection of biomarkers is based on gene expression profiling or protein profiling to distinguish the cancer and normal samples. The analysis in this phase usually characterized by ranking and finding suitable ways to combine biomarkers.
Phase 2	After completion of phase 1 an assay is established based on the clinical use. The clinical assays could be a protein, RNA-DNA or cell based techniques including ELISA, PCR, gene arrays etc. these assays should be evaluated for clinical performance in terms of Sensitivity and specificity.
Phase 3	In this phase investigator evaluates the sensitivity and specificity for clinical detection of diseases and analysis including follow-up to verify occurrence of disease.
Phase 4	In phase 4 false referral rates can be estimated by investigators based on tested biomarkers to describe the extent and characteristics of disease detected.
Phase 5	In this phase evaluation for benefits and risks of new diagnostic tests are verified for biomarker screening and detection.

Applications of Biomarkers as an Emerging Tool

Biomarker in Disease Diagnosis

Biomarkers depicting prodromal signs enable earlier diagnosis or allow for the outcome of interest to be determined at a more primitive stage of disease. Urine, blood, and cerebrospinal fluid provide the necessary biological information for the diagnosis. In these circumstances, biomarkers are used as an indicator of a biological factor that represents either a subclinical aspect, stage of the disorder, or a surrogate manifestation of the disease. The application of biomarkers in the diagnosis and management of infections, cardiovascular disease, immunological and genetic disorders, and cancer are well known[2]. For example, the

cytokeratin-18 fragment level as a biomarker of non-alcoholic fatty liver disease in patients with type 2 diabetes mellitus article highlights that the CK-18 level was the best independent determinant of NAFLD in T2DM patients. The CK-18 level was associated with the AST and ALT levels in the T2DM patients. The change in the CK-18 levels was considerably correlated with the changes in BMI. The CK-18 is a potentially useful biomarker for NAFLD in patients with T2DM[12]. Cardiovascular diseases are the most prominent circulation disorders around the world. Biomarkers of inflammation and oxidative stress may serve to monitor the efficacy of treatments, to help identify patients at risk for CVD, and to evolve new pharmacological tools. The identification of the most appropriate set of biomarkers can provide a detailed picture of the specific nature of the cardiovascular event. Cardiac markers are biomarkers measured to evaluate heart function. Most of the early markers identified were enzymes, and as a result, the term "cardiac enzymes" is occasionally used. However, not all of the markers directly used are enzymes. Examples of some cardiac markers are lactate dehydrogenase, Troponin, etc. Lactate dehydrogenase catalyses the conversion of pyruvate to lactate. LDH-1 isozyme is typically found in the heart muscle and LDH-2 is found predominately in blood serum. Higher LDH-1 level to LDH-2 proposed MI. Troponin is released during MI from the cytosolic pool of the myocytes. Its successive release is prolonged with degradation of actin and myosin filaments. Isoforms of the protein, T and I, are specific to myocardium. Differential diagnosis of troponin elevation includes acute infarction, serious pulmonary embolism causing heart failure, myocarditis, acute right heart overload. Infarct size can be calculated by troponin but the peak must be measured in the 3rd day. After myocyte injury, troponin is released in 2–4 hours and persists for up to 7 days. The biomarkers of Alzheimer's disease (AD) are required to distinguish between dementia and normal aging, dementia and one disorder from another, as well as to compensate in diagnosing the exact cause of a dementia. Thus, AD biomarkers, and other neurodegenerative disorders such as Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD), must be reliable and distinct, and they should be useful in managing us to make more accurate diagnosis and better treatment of the diseases. The CSF biomarkers like hyperphosphorylated tau (P-tau), total tau (T-tau) and the 42 amino acid isoform of amyloid β (Aβ42) reflect the core pathologic features of AD, which are intracellular neurofibrillary tangles neuronal loss, and extracellular senile plaques. After all the pathological mechanism of AD start decades before the first symptoms, these biomarkers may contribute the means of early disease detection. Parkinson's disease (PD) is the second most common neurodegenerative disorder and the search for such biomarkers is under process. For example, examination of ligand uptake using neuroimaging techniques which reflect nigrostriatal tract integrity are now available in the clinical and research realms and other imaging modalities are in active testing[13,14].

Biomarkers in Drug Development

The role of biomarkers has been exponentially increasing in guiding decisions in every phase of drug discovery, drug development and preclinical interpretation through each phase of clinical trials and into post-marketing studies. In early phases of drug development, biomarkers are used to evaluate activity in animal models, prove mechanism of action and concept of an investigational entity, bridge pre-clinical and clinical pharmacology, and interpret safety in animal models and humans. In late stages of drug development, biomarkers can be used to make decisions in the evaluation of dose-response and optimal regimen for desired pharmacologic effect and safety. They are now becoming more and more integrated into all stages of the development process, ranging from target discovery, evaluation of drug activity, understanding mechanisms of action, toxicity and safety evaluation, internal decision making, clinical study design, diagnostic tools, understanding disease processes. PD biomarkers are commonly observed to respond proportionally with dose. Safety molecular

biomarkers have been used for decades both in preclinical and clinical research. Among the most common safety tests are those of liver function (e.g., transaminases, bilirubin, alkaline phosphates, etc.) and kidney function (e.g., serum creatinine, cystatin C, creatinine clearance). Others include markers of skeletal muscle (e.g., myoglobin) or cardiac muscle injury (e.g., CK-MB, troponin I or T), as well as bone biomarkers (e.g., bone-specific alkaline phosphates). Biochemical and molecular markers have revolutionized medicine and drug development in recent decades. For example, the blood of HIV patients can be tested for its viral load to assess the course of their disease, further providing a surrogate endpoint for trials of anti-HIV drugs. Application of biomarkers in the development of drugs intended for the treatment of Osteoarthritis, the goal of this document is to provide a summary and guide to the application of in vitro (biochemical and other soluble) biomarkers in the development of drugs for OA. This document compiles definitions and classification systems for biomarkers, the present outcome measures used in OA clinical trials, operations and possible efficacy of biomarkers for development of OA analysis, the recent state of accomplishment of OA-related biomarkers, pathways for biomarker qualification, demanding needs to advance the use of biomarkers for drug development, and a research agenda to advance the science of OA-related biomarkers[2,5,6,15].

Biomarkers in Disease and Treatment

- Markers of disease in Prostate Cancer: There are no reliable biomarkers for disease progression in aggressive prostate cancer that has demonstrated utility in product development. Although prostate specific antigen (PSA) is used for a variety of purposes (e.g., determining when further diagnostic testing is indicated, assessing response to therapy), there is no consensus on how best to use PSA in cancer therapeutic trials. Uses of PSA that should be further investigated including identifying high risk populations, arranging an early marker of drug action and dose range, and use of PSA as a marker of disease progression. A gap analysis to rigorously identifying what is proven and unproven about PSA and other potential indicators would be an important first step in improving prostate cancer biomarkers[2].

- Biomarkers in Arthritis: The well-known rheumatoid factor (RF) and the anti-citrullinated protein antibodies (ACPA), several new markers are now likely to become available with interesting possibility. Likewise, antibody responses controlled against citrullinated proteins, also antibodies across carbamylated proteins (anti-CarP) have recently been shown to be current in Rheumatoid Arthritis (RA). Fascinating these anti-CarP antibodies are also present in around 20% of the ACPA-negative RA patients and are associated with more severe joint damage in this group. MRI has demonstrated promise for detecting soft tissue inflammation and cartilage erosion in rheumatoid arthritis. If set as a reproducible biomarker, use of MRI could help resolve the potential of a new therapeutic product, stratify patients by risk and identify dose ranges while serving as an early response measure[2,16].

- Biomarkers in Chronic Obstructive Pulmonary disease: The natural history of chronic obstructive pulmonary disease (COPD) is marked by episodes of deterioration, called exacerbations of COPD (ECOPD) which lead to increased morbidity and mortality. In addition to the imaging and functional markers, sampling can provide an insight in the pathophysiological mechanisms of ECOPD. Elaborated sampling techniques offer an innovative basis for the identification of pulmonary biomarkers. These approaches may be totally non-invasive [e.g. exhaled air, exhaled breath condensate (EBC), spontaneous sputum (SS)], semi-invasive (e.g. induced sputum (IS), nasal wash, large airways' secretions) or invasive [e.g. bronchoalveolar lavage (BAL)]. Measuring biomarkers in the breath is a very attractive approach to monitoring COPD inflammation, as it is non-invasive and makes repeated

sampling possible. COPD patients with pulmonary hypertension and corpulmonale may have lower FENO levels, perhaps due to impaired endothelial release of this vasodilator mediator, and this might help identify those COPD patients. Exhaled breath condensate (EBC) is collected by cooling or freezing exhaled air. Borrill et al. explored the potential of EBC mediators to be used as biomarkers in COPD. For certain EBC mediators, especially cytokines, the ELISA method has been correlated with inadequate assay sensitivity and specificity. COPD patients exhibit a very low pH, of exhaled breath condensate which may be indicative of a specific COPD phenotype. Collection and analysis of sputum is a commonly used non-invasive means of assessing airway inflammation in COPD. An increase in the percentage of neutrophils in sputum is the typical feature in subjects with COPD. BAL allows examination of the disease process in situ by allowing the sampling of metabolites deep in the tissue at the level of bronchioles and alveolar ducts.

Lymphocytes are generally higher in ex-smokers than in smokers, whether with or without COPD. However, the percentage of CD8+ T lymphocytes is significantly higher, and that of CD4+ T-cells significantly lower, in COPD (and healthy smokers) compared with healthy non-smokers. Bronchial biopsies have been useful for documenting the structural changes, cellular patterns and expression of inflammatory proteins in patients with COPD.

In effect, biopsies give reliable histopathological information on the inflammatory state within the bronchial tissue that is independent of factors that may affect sputum and BAL samples, such as processing and dilution issues. This is the only technique that directly samples the resident cells and maintains the spatial relationships with structural components. Therefore, the information is additional and complementary to that obtained by lavage and sputum[17].

Biomarkers in Assistance with Imaging Technologies

Imaging-based biomarkers employ a variety of technologies to capture images of anatomical and physiological changes in the body. Imaging biomarkers have many advantages:

- They usually non-invasive and they produce spontaneous, multidimensional results.
- Yielding both qualitative and quantitative data, they are comfortable for patients.
- Biomarkers serve as useful sources of information to the clinicians seeking to make a diagnosis when combined with other sources.

One example of an imaging biomarker in the clinic is the use of the fluorine isotope combined with the glucose analogue fludeoxyglucose ((18F) FDG) in PET/CT to diagnose reoccurrence of tumour in colon cancer. Served as surrogate of glucose metabolism, PET/CT imaging is crucial in [detecting colon cancer recurrence] compared to biochemical markers because you need localization of the recurrence to offer surgery, the only curative treatment in these type of patients.

1. *X-Ray:* Multiple DNA and protein biomarkers have been detected based on characteristic x-ray fluorescence of a panel of metal and alloy nanoparticles, the description and measure of biomarkers can be detected with limits of 1 nM for DNA and 1 ng/ml for protein by determining the presence and concentration of nanoparticles using x-ray fluorescence. Quantitative imaging of multiple biomarkers is possible by coupling high penetrating capability of x-rays.
2. *Computed tomography:* Sometimes also called computed axial tomography (CAT scan), in this technique x-rays are used to take a series of 2-dimensional images which are then digitally converted to a 3-dimensional image. There has been tremendous increase in developing novel pulmonary biomarkers to assist in drug and device development in the setting of patients

with chronic obstructive pulmonary disease and diffuse parenchymal lung disease.

3. *Magnetic Resonance Imaging (MRI):* Radio frequency signals are used to alter the atoms' magnetic alignment and the resulting signal is detected by scanners. MRI is more appropriate than tomography in distinguishing soft tissues. Two MRI methods that have already been incorporated into clinical trials of treatment response in solid tumours: Diffusion imaging and Dynamic contrast-enhanced MRI. The homogenous high signal intensity of upper tract urothelial carcinoma and bladder cancer on diffusion-weighted magnetic resonance imaging provides helpful diagnostic information for the presence of cancerous lesions in a non-invasive manner.

4. *Positron Emission Tomography (PET):* Positron Emission Tomography (PET), as a non-invasive imaging technique that provides quantitative information about a drug target's circulation, its communication with drug molecules and changes with time and therapeutic intervention, has been progressively identified as a powerful imaging technique that provides a specific and sensitive biomarker for drug development, especially the development of drugs meant for targeting the CNS. PET imaging uses biologically active compounds labelled with positron-emitting radioisotopes such as carbon-11 (decay half-life of 20.4 min) or fluorine-18 (decay half-life of 109.8 min). The radio labelled compounds also called as radiotracers, or radiopharmaceuticals is inserted into a research subject lying in the scanner. Positron (an anti-electron) emitted by the isotope travels a short distance before striking an electron. The collision eradicates the two particles and emits two gamma rays travelling in opposite directions which are detected by a scanner. Computerized tomography assembles a 3-dimensional image of the area of interest. The first PET machines for use in humans were introduced in early 1970. Amyloidal content assessments by PET scan may be useful imaging techniques to demonstrate the effect of potential Alzheimer's therapies[2].

CONCLUSION

Biomarkers basically act as indicator of a particular disease or biological state of an organism and offer means for uniform classification of disease and risk factors. Biomarkers have become an essential part of clinical development because they offer a faster alternative to the conventional drug development approach and the promise of safe drugs, in large numbers, accepted more quickly is achieved. Biomarkers help in understanding the spectrum of chronic disease with application as an emerging tool in drug development, screening, diagnosis, prognosis, prediction of recurrence of disease and therapeutic monitoring. Advances in genomics, proteomics and molecular pathology have generated many candidate biomarkers with potential clinical value. The use of biomarker will possibly increase the cost of clinical development and in the long term their use should lower the cost and duration of clinical development.

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